Early Detection Research Network Specimen Reference Sets

Biomarker Reference Sets for Cancers in Women (BRSCW)

Information

The Biomarker Reference Sets for Cancers in Women (BRSCW) are three sets of serum specimens for initial phase I evaluation of potentially hundreds of new biomarkers to assess their usefulness in the early detection of breast, endometrial, ovarian, or other female cancers using identical specimen sets. The common feature of all three sets is that they include individual aliquots from 95 healthy women with no personal or strong family history of cancer and 20 identical replicates (for assessing an assay's coefficient of variation). These specimens were collected using the facilities of a blood donation laboratory permitting large volume blood draws that allowed creation of 275 serum aliquots of 0.3ml size for all of the controls and an additional serum pool for creating identical replicates.

Because large volume blood draws cannot be safely performed in women coming to surgery for a possible cancer, we have chosen to combine a larger number of smaller aliquots to create cancer or benign disease pools to be included in 2 of the three sets. Pooling is a reasonable method for initial evaluation of a biomarker in case or control specimens when an assay is costly or the number of specimens on individual cases or controls is limited (1). An investigator should be able to gain an initial estimate of the value of a biomarker by examining where, in relation to the actual distribution of the biomarker in the controls, the overall value for a pooled specimen falls. The standard markers (CA125, CEA, CA72.4, CA15.3, and CA19.9) have been measured in one complete set of specimens so that comparison of a new marker with standard markers will be possible. A new biomarker, showing no better performance than an existing marker, would have lower priority for moving forward.

Three BRSCW sets have been constructed. BRSCW1 consists of 115 individually-barcoded plastic straws holding 0.3ml of sera from 95 female control subjects (see Table 1 for description of the controls) and 20 straws that contain identical aliquots of pooled control sera for assessing the coefficient of variation (CV) of the assay. It is anticipated that these sets would be useful for the investigator who has already assessed a marker in women with cancer other than breast, endometrium, or ovary and needs a well-annotated set of control specimens in women for comparison. 140 of these sets are initially available.

Table 1 Description of Control Subjects in BRSCW:

Characteristic	N (%)	Characteristic	N (%)
Age		Ever pregnant?	
<30	5 (5%)	Yes	68 (72%)
30-39	12 (13%)	No	27 (28%)
40-49	25 (26%)	Ever take birth control pills?	
50-59	39 (41%)	Yes	60 (63%)
≥60	14 (15%)	No	35 (37%)
Race		Ever use HRT?	
White	79 (83%)	Yes	26 (27%)
Black	11 (12%)	No	68 (72%)
Other and Unknown	5 (5%)	Unknown	1 (1%)
Menopausal status		Ever Smoked?	
Pre-menopausal	39 (41%)	Yes	44 (46%)
Post-menopausal	56 (59%)	No	51 (54%)

BRSCW2 consist of 122 plastic straws including the 115 straws described above plus 7 additional straws containing sera from pools that represent benign and malignant pelvic disease. These 7 pools were constructed

from blood collected pre-operatively from premenopausal or postmenopausal women with endometrial or ovarian cancer and benign gynecologic diseases as described in Table 2. It is anticipated these sets would be useful for investigators who wish to assess biomarkers for endometrial or ovarian cancer but not breast cancer. They will also be given out after all of the BRSCW3 sets that contain the complete set with breast, endometrial, and ovarian cancer are used up. 20 of the BRSCW2 sets are initially available.

Tables 2: Ovarian/Endometrial Disease Pools (# women contributing to pool)

1. Premenopausal women with endometriosis (38)
2. Postmenopausal women with benign serous ovarian tumors (35)
3. Premenopausal women with late stage, non-mucinous ovarian cancer (35)
4. Postmenopausal women with late stage, non-mucinous ovarian cancer (39)
5. Postmenopausal women with early stage, non-mucinous ovarian cancer (35)
6. Pre-/postmenopausal women with mucinous ovarian cancer (35)
7. Pre-/postmenopausal women with endometrial cancer (12)

The BRSCW3 sets contain 127 straws including the 122 described for BRSCW2 above plus 5 additional straws containing sera from pools that represent benign, pre-invasive, and invasive breast disease (Table 3). 115 of the BRSC3 sets are initially available.

Table 3: Breast Disease Pools (# women contributing to pool)

1. Premenopausal women with benign breast disease (45)
2. Postmenopausal women with benign breast disease (45)
3. Premenopausal women with invasive breast cancer (43)
4. Postmenopausal women with estrogen receptor positive invasive breast cancer (36)
5. Pre-/postmenopausal women with DCIS (43)

The BRSCW sets were constructed through a collaboration between the Partner's Southwestern Clinical Epidemiology and Validation Center and the Duke Biomarker Development Lab. An automated "MAPI" system (Cryo Bio System, Paris, France) was used to aliquot and label the straws. Labels on the straws are randomly ordered; and investigators cannot link the ID to the specimen type (an individual control, a replicate, or cancer or benign disease pool). Only when the results are returned to the Data Management and Coordinating Center, that holds the key, can the results be interpreted. The sets are stored in containers called "Goblets" in liquid nitrogen at the NCI Fredericks Facility.

The specimens in this study were collected under protocols that allowed for sharing with qualified EDRN investigators. Clearly the pooled case specimens and replicate control specimens cannot be associated with any individual subject. The identity of the control subjects has been retained by the commercial blood bank and is unknown to Partner's Investigators. Thus, the ability to link the specimen to a specific individual is impossible for the investigator receiving the specimen; and it has been determined that the specimens are "exempt" from the need for human subjects approval for the recipient investigator. However, investigators may wish to obtain a ruling from their local IRB using this description of the specimen set.

To be eligible to receive one of the BRSCW sets, investigators must complete the "BRSCW Application Form" attached and receive approval from the appropriate EDRN Collaborative Group and the Executive Committee. This form requires the investigator to demonstrate that a workable assay has been developed for use on sera and that preliminary data is available that the marker may have value in the detection of breast, endometrial, ovarian cancer, or a cancer other than these (if only control specimens are desired). In addition the investigator must show that resources are available to process the specimen and agree to certain conditions including the timely return of the data to the DMCC and posting of the results on the EDRN website.

1. Weinberg, CR and Umbach DM. Using Pooled Exposure Assessment to Improve Efficiency in Case-Control Studies. Biometrics 1999;55:718-726

Early Detection Research Network

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Application Information

Investigator and Shipping Information

Investigator Name:
Institution:
Address:

Phone Number:
Fax Number
Email address:

Contact Person responsible for receipt of specimens:
Name:
Institution:
Address:
Phone Number:
Fax Number:
Email address:

Cancer, Marker, and Reference Set

Women's Cancer of particular interest?_____

Name or family of markers being assessed? _____

Which set is requested? ☐ BRSCW1 ☐ BRSCW2 ☐ BRSCW3

Background for linking Biomarker to Cancer

Describe in no more than 500 words the preliminary data which links the marker(s) with the cancer of interest. Up to five references may be included.

Assay Characteristics

- Describe the nature of the assay (e.g. ELISA with polyclonal or monoclonal)

- What target analyte and related proteins does the assay measure?

- Characterize the calibrators
  - Description:_____ [purified from?, recombinant, synthesized]
  - Species: _____[human, etc]
  - Purity:_____
  - Units:_____

- Dynamic range of the assay?
  - Limit of detection:_____
  - Limit of quantitation: (20%CV)_____
  - Within Assay variance (%CV)____
  - Between assay variance (% CV)_____
  - Most measurable without dilution_____

- Assay Robustness:
  - Temperature stability (time/temp)_____
  - Freeze/thaw stability (# F/T cycles)_____

- Is the analyte measurable at a level with adequate precision in serum?
  - Sera tested: (normal male, tumor pt, etc.)_____
  - RO range observed:

Conditions

	Yes	No
➢ Are funds currently available to cover the costs of performing the assay?	☐	☐
➢ <u>Does the investigator agree to the following conditions</u>:		
• I agree not to resell or release the BRSCW set or sub-aliquots from this set to an investigator not directly connected with this application.	☐	☐
• I agree to complete the assays on the BRSCW specimens within 2 months of their receipt	☐	☐
• I agree to return assay results to the DMCC within 3 months of receipt of specimens	☐	☐
• I agree to release assay results for posting on the EDRN website within 2 months after I have received the unblinded results back from the DNCC for my review.	☐	☐

Signature

Date